# MafB is essential in macrophages for regulating Brown Adipose Tissue's cold-induced Neuronal arborizations

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| 著者（英） | Kumar Yadav Manoj |
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| 氏　　名 | Manoj Kumar Yadav |
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| | （職名） | （学位） | （氏名） |
|---|---|---|---|
| 主　査 | 筑波大学教授 | 医学博士 | 島野　仁 |
| 副　査 | 筑波大学教授<br>（グローバル教育院） | Ph.D. | Kim. Seong-Jin |
| 副　査 | 筑波大学教授<br>（グローバル教育院） | 医学博士 | 佐藤　孝明 |
| 副　査 | 筑波大学准教授 | 博士（医学） | 渋谷　和子 |
| 副　査 | 筑波大学教授 | 医学博士 | 高橋　智 |

# 論文の要旨
## Abstract of thesis

**Background**: Obesity becomes an epidemic disease in modern times. The population of theoverweight raised up to 39 % as per the World Health Organization(WHO) 2016 report. Interestingly, the WHO data indicates the countries are having a higher prevalence of obesity mostly have a cold climatic environment. Brown Adipose Tissue's (BAT) metabolism has emerged as one of the promising solutions for burning extra calories and could be a solution to combat obesity in cold-climatic countries. In recent developments, Adipose Tissue Macrophages (ATM) is indicated to plays a critical role in BAT's neuronal density regulations. However, the mechanism was not fully understood.  In this current study, the applicant attempted to unveil the possible role of ATM during cold adaptation and neuronal plasticity in BAT.
Thus, he hypothesized that "MafB (Musculoaponeurotic Fibrosarcoma Homolog B gene), a bZip transcription factor that plays an important role in the macrophages differentiation & functions, might also have a possible role in cold-induced thermogenic pathways including modulation of neuronal density."

**Aim**: He set the aim of the project to unveil the role of MafB in macrophages during cold induced-thermogenesis.

**Methods**: To make cold acclimatization, he did ten days continuous cold exposure at 4°C or Intermittent cold exposure at 8°C-8 hours alternate days for one week, to $Mafb^{f/f}$ transgenic mouse which has floxed Mafb gene alleles (CL57BL/6J genetic background) as control wild type or macrophage-specific Mafb deficient $Mafb^{f/f}$-$Lysm^{Cre}$ (Mafb-Mf-CKO) mice. On the tenth day of cold exposure at 4°C or the eighth day of intermittent cold exposure, he performed the required experimental analysis.

**Results**: He obtained the following major results:
1. Mafb expression induced in BAT during cold exposure: After intermittent cold acclimatization of wild type mice's BAT, RT-PCR results show increased Mafb expression. This suggests a possible role of Mafb during cold adaptation.
2.Mafb-Mf-CKO mice are deficient in cold-induced thermogenesis: Mafb-Mf-CKO mice after acute cold challenge shows a significant drop-down of their rectal temperature with comparison to control. The ten days continuous cold exposure at 4°C results in a significant increase in the bodyweight of Mafb-Mf-CKO mice with a comparison to control with a significant dropdown of rectal temperature by 0.5°C. Moreover, Mafb-Mf-CKO mice b3 agonist-induced BAT's thermogenic activity is measured by calorimetric analysis VO2/VCO2, and results observed were a significant decrease in energy expenditure in Mafb-Mf-CKO.
3.BAT functions of Mafb-Mf-CKO mice are compromised: He analyzed the BAT's function by RNA sequencing and RT-PCR, and the results observed decreased in Ucp1 expression with a decrease in BAT functional genes. Similar to RT-PCR data Ucp1 staining by IHC shows a significant decrease. Moreover, Hematoxylin and Eosin Staining shows a bigger lipid droplet in the Mafb-Mf-CKO mice BAT. This data suggest a decrease in the function of BAT of Mafb-Mf-CKO mice.
4.Abnormal cytokine production in Mafb-Mf-CKO mice: 24-hour short cold exposure of Mafb-Mf-CKO mice showed an increase in Il6 proinflammatory cytokine, which is further confirmed by ELISA. Previous studies indicate Il6 is suppressed by Mafb, while Il6 induction can affect the Neuron Growth Factor (Ngf). This suggests during adaptation increase in Il6 could affect cold-induced neuronal density plasticity.
5.Mafb-Mf-CKO mice impaired for the cold-induced increase in neuronal density of BAT: IHC analysis by using Th (Tyrosine Hydroxylase) antibody, a sympathetic neuron marks clearly depicts a decrease in neuron density of BAT of Mafb-Mf-CKO mice.
6.Il6-Receptor antibody treatment toMafb-Mf-CKO mice rescued the phenotype: He rescued the Mafb-Mf-CKO mice by injecting the Il6-receptor antibody during acclimatization to the cold and analyzed by phenotypic analysis. The rectal temperature, body weight, VO2/VCO2 has improved with comparison to the PBS treated group.

Conclusion: Based upon these results, he concluded that Mafbin macrophages plays an essential role during the cold adaptation of the BAT mediated by Il6 cytokine which affects the neuronal density of BAT.

## 審査の要旨
## Abstract of assessment result

【批評 Review】
It is a novel and implicative piece of work that macrophages are increased and activated in brown fat tissues upon cold exposure, where the applicant clearly showed MafB plays a critical role in thermogenesis. His hypothesis: cold exposure-induced thermogenesis requires MafB in some specific subtypes of macrophages which suppresses IL-6 secretion and de-dysregulate neuronal arborization at BAT, is a very intriguing story, but complex and involves spatio-temporary interactions among BAT, macrophages, peripheral nerves and others, which needs to be precisely elucidated using a comprehensive technology such as single cell transcriptome. The concept would open a new therapeutic strategy for obese-related problems.

【最終試験の結果 Result】
The final examination committee conducted a meeting as a final examination on 20 January,2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A

session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】
Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.